

actuator **372** is positioned such that the valve cavity **374** is in fluid communication with the upper receptacle **320** prior to use. The device contains approximately 10 mg of autoclaved CM-111 3M Cosmetic Microspheres in the upper receptacle **320**. Lower receptacle **324** contains a liquid detection reagent **365**, which consists of approximately 0.6 milliliters of the luciferase/luciferin liquid reagent solution from a Clean-Trace surface ATP system. BARDAC 205M beads are made according to Preparative Example 5 of U.S. Patent Application No. 61/101,546, filed Sep. 30, 2008. Ten milliliters of sterile deionized water is added to the upper receptacle **320** of the unitary devices **300** immediately before use.

[0208] *E. coli* overnight cultures are prepared as described in Example 2. The bacterial culture is diluted to approximately 10^6 or 10^5 CFU/ml in Butterfield's buffer. One hundred microliters of the diluted suspension are pipetted directly into upper receptacle **320** of the unitary devices **300** to obtain a suspension of approximately 10^5 CFU or 10^4 CFU in ten milliliters, respectively. The cap **378** is used to close the housing **310** and the bacterial suspension is mixed with the microspheres (cell concentration agent **330**) at room temperature and allowed to settle into the valve cavity **374**. The cap **378** is removed and two BARDAC 205M beads (hydrogel **362**) are dropped into the housing **310**. Immediately after the beads settle into the valve cavity **374**, the valve actuator **372** is turned to transfer the portion of the liquid sample in the valve cavity (containing the cell concentration agent **330** and the hydrogel **362**) into the lower receptacle **324** containing the ATP detection reagents. The unitary device is immediately inserted into the reading chamber of a luminometer (for example, a NG Luminometer, UNG2) and RLU measurements are recorded at 10 sec interval using the Unplanned Testing mode of the UNG2 luminometer. RLU measurements are collected until the number of RLUs reaches a plateau. The data are downloaded using the software provided with the NG luminometer. The data will indicate that the microbial cells are concentrated by the microspheres, the cell extractant is released by the hydrogel, the cell extractant causes the release of ATP from the cells, and the ATP released from the cells is detected by the ATP detection system.

Example 5

Preparation of Detection Devices

[0209] Type I devices: For these detection devices, housings similar to the housing of FIG. 10A were constructed with the differences noted below. Reference numbers below refer to the corresponding parts in FIG. 10A. The upper parts **1012** and lower parts **1014** of the housing **1100** were obtained using the analogous components from 3M Clean-Trace™ surface ATP tests (obtained from 3M Company, Bridgend, UK). A collector **1067** with a frangible seal **1068** coupled thereto was press-fit into the upper portion of the lower part **1014**; with the frangible seal **1068** facing the lower part **1014** of the housing **1100**. The upper part **1012** was coupled to the lower part **1014** using a 2 cm section of 3:1 polyolefin dual wall adhesive lined heat shrink film obtained from buyheatshrink.com (part #_HSC3A-050-cc, 1.5 cm in diameter) using a heat gun (Master Appliances Corp, Racine, Wis.).

[0210] For these detection devices, plungers similar to the plunger of FIG. 2A were constructed. Reference numbers below refer to the corresponding parts in FIG. 2A. The plunger (**250**) was assembled using a portion of the polyolefin plastic handle (**252**) from a 3M Clean-Trace™ surface ATP

test, a brass metal shaft (**251**) and an acetal piercing member **259**. The handle **252** and piercing member **259** were attached to the ends of the brass shaft via threaded connections. The brass metal shaft was 11.5 cm long and 3.9 mm in diameter. A 6 mm, 6-23 thread was produced on each end of the shaft using a lathe. The piercing member **259** was fabricated from 1/2-inch (12.7 mm) acetal copolymer rod (part number 8497K211, obtained from McMaster-Carr, Santa Fe Springs, Calif.) using a 10" Southbend lathe. An O ring (Buna N AS568A Dash Number 010 obtained from McMaster-Carr) was used as the lower seal **256** and was attached to the plunger **250** approximately 11.5 mm above the piercing end **259**. The plunger was surface-sterilized before each use.

[0211] Type II devices: These detection devices were assembled using a plunger similar to that shown and described in FIG. 5A with a tip similar to that shown in FIG. 6A. The housing was constructed as described for the Type I devices. The tip of the plunger was fabricated from 1/2-inch (12.7 mm) acetal copolymer rod (part number 8497K211, obtained from McMaster-Carr, Santa Fe Springs, Calif.) using a 10" Southbend lathe. The a duckbill one-way valve and a plastic retaining washer were press-fit into the recessed opening of the body of the tip of the plunger. The filter was made by machining a POREX filter (part number X6854 from Porex Corporation, Fairburn, Ga.) to the shape shown in FIG. 6A and dimensioning one end to press-fit into the recessed opening of the tip and hold the valve and retaining washer in place. The plunger was surface-sterilized before each use.

[0212] Type III devices: Detection devices similar to those shown in FIG. 10A were constructed with the differences noted below. Reference numbers below refer to the corresponding parts in FIG. 10A. The upper parts **1012** and lower parts **1014** of the housing **1100** were obtained using the analogous components from 3M Clean-Trace™ surface ATP tests (obtained from 3M Company, Bridgend, UK). A collector **1067** with a frangible seal **1068** coupled thereto was press-fit into the upper portion of the lower part **514**; with the frangible seal **1068** facing the lower part **1014** of the housing **1100**. The upper part **1012** was coupled to the lower part **1014** using a 2 cm section of 3:1 polyolefin dual wall adhesive lined heat shrink film obtained from buyheatshrink.com (part #_HSC3A-050-cc, 1.5 cm in diameter) using a heat gun (Master Appliances Corp, Racine, Wis.).

[0213] The plunger (**1050**) was assembled using a portion of the polyolefin plastic handle (**1052**) from a 3M Clean-Trace™ surface ATP test, a brass metal shaft (**1051**) and tip **1090**. The handle **1052** and tip **1090** were attached to the ends of the brass shaft via threaded connections. The brass metal shaft was 11.5 cm long and 3.9 mm in diameter. A 6 mm, 6-23 thread was produced on each end of the shaft using a lathe. The tip **1090** was fabricated from 1/2-inch (12.7 mm) acetal copolymer rod (part number 8497K211, obtained from McMaster-Carr, Santa Fe Springs, Calif.) using a 10" Southbend lathe. An O ring **1086** was attached to the tip **1090**. The tip was machined to include a retaining member **1087**, as shown in FIG. 10. A scraper was constructed by die-cutting a piece of 1 mm-thick polyurethane rubber and slipping it into the retaining member **1087**. The outer diameter of the scraper **1086** was dimensioned to provide a tight fit with the inside of the housing **1010**. The plunger was surface-sterilized before each use.